Several specific mechanisms can be envisioned for these quasi-linear domains, which remarkably are distributed near the radial paths of microtubules. Is there a linear path cut through the actin-bound membrane fences (Figure 1A)? If so, the confinement width should broaden when the cortical actin meshwork is disrupted. Do microtubules peel the cortical actin away from the bilayer and thus create a "tent" of actin-free membrane (Figure 1B), or do they preferentially localize to the well-known folds of macrophages? This should be discernable in electron tomography. Is actin-binding transmembrane protein (ABTP) density spatially dependent on both actin and tubulin (i.e., a scaffolded picket fence; Figure 1C)? In this scaffolded picket fence model, microtubules just below the membrane could break the connections between cortical actin and the ABTP "pickets" in the membrane, clearing a path for diffusion of membrane components along a channel but leaving the cortical actin itself intact and still in reasonable proximity to the membrane for rapid reversal of tubulin-dependent effects. Is the confinement zone itself moving within the membrane, either by diffusion or by attachment to one or more molecular motors (Figure 1D)? In this case, the diffusion would be microscopically isotropic with confinement to 190 nm, but a nanoscale drift in one direction would be superimposed, as, for example, in the Smoluchowski equation (Berezhkovskii et al., 1989). There is precedence for the model of cytoskeletal movement, such as in the radial actin motoring in the immune synapse (Yu et al., 2010). Further experiments will be necessary to fully elucidate the mechanism behind these domains.

The capabilities of superresolution microscopy and other single-molecule techniques may be the key to understanding how directed motion (for example via actomyosin and microtubules) is cooperatively coupled to diffusive motion within the bilayer. Simultaneous three-dimensional superresolution live cell imaging of actin, tubulin, and membrane components, for example, could potentially distinguish directly between the models discussed here. The techniques used by Jaqaman et al. to measure directly collision rates and metastable clustering elegantly add the important fourth dimension of protein-protein interaction missing from most membrane domain studies. Future studies using these techniques will be needed to test the hypothesis that the geometry per se of cytoskeletal confinement plays a significant physiological role for cell signaling.

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Meeting the (N-Terminal) End with Acetylation

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Cell-fate decisions are tightly linked to cellular energy status. In this issue, Yi et al. (2011) introduce a mechanism by which Bcl-xL lowers the threshold for apoptosis by suppressing acetyl-CoA production, which, in turn, suppresses the N-alpha-acetylation important for activation of the proapoptotic protease caspase-2.

A cell's decision to die by apoptosis, become quiescent, or proliferate is influenced by the metabolic conditions of the cell and its surrounding tissue. Over the past decade, multiple studies have established direct links between these critical cellular pathways. For example, when glucose levels fluctuate, glucose-sensing pathways transduce signals through the

AKT kinase to alter the post-translational modifications and/or expression levels of Bcl-2 family proteins (reviewed in Buchakjian and Kornbluth, 2010). More challenging, given the vast array of metabolites in the cell and the tangled metabolic circuitry into which they feed, has been the elucidation of interactions between small-molecule metabolites and cell-fate signaling pathways. Yi et al. (2011) now demonstrate that the antiapoptotic potency of Bcl-xL protein stems in part from its ability to lower levels of acetyl-CoA, a substrate of protein acetyltransferases (Figure 1). This results in the decreased N-alpha-acetylation of multiple apoptotic regulators, including caspases and the proapoptotic Bcl-2 family member Bax, thereby increasing resistance to apoptotic stimuli.

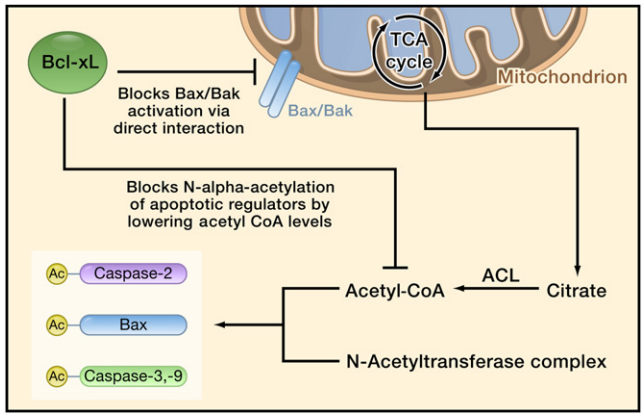

Figure 1. Regulation of Apoptosis by Bcl-xL
In addition to directly blocking Bax/Bak oligomerization and cytochrome *c* release from the mitochondria, Bcl-xL also lowers the levels of acetyl-CoA (possibly due to an indirect effect upstream of acetyl-CoA), resulting in decreased N-alpha-acetylation of apoptotic mediators such as caspase-2, -3, -9 and Bax. In the case of caspase-2, N-alpha-acetylation is critical for its activation.

Distinct enzymes control protein N-terminal acetylation and the acetylation of lysine residues. Lysine acetylation is increasingly appreciated as a dynamic signaling event, but the role of N-alpha-acetylation has been more enigmatic. N-alpha-acetylation is catalyzed by N-terminal acetyltransferases (NATs—comprising NatA through NatF in eukaryotes) and is thought to aid in the proper function, localization, and stabilization of certain proteins (reviewed in Arnesen, 2011). However, N-alpha-acetylation is considered irreversible, and early studies showed that most eukaryotic proteins have this modification, thus branding it as a global modification required for many proteins to function. The findings of Yi et al. suggest that N-alpha-acetylation may have more nuanced and varied biological roles.

To monitor protein N-alpha-acetylation, the authors use a clever strategy that involves subtiligase-mediated biotinylation and avidin capture of only those proteins with free N termini. These experiments reveal that the N-alpha-acetylation of apoptotic regulators is sensitive to acute changes in the levels of acetyl-CoA, suggesting that a cell's threshold for death is intimately linked, via this modification, to nutrient status.

The idea that a posttranslational modification like N-alpha-aceytlation is regulated by levels of the donor substrate, acetyl-CoA, rather than by the enzymes that catalyze the modification runs counter to common wisdom concerning other modifications like phosphorylation, where the phosphoryl donor ATP is typically not limiting. Interestingly, a small but growing body of literature suggests that protein acetylation is indeed influenced by fluctuating acetyl-CoA levels. For example, glucose-mediated stimulation of acetyl-CoA production via citrate/ATP citrate lyase results in increased histone acetylation and consequent transcription of metabolic regulators (Wellen et al., 2009). These observations and those by Yi et al. imply that changes in a single metabolite, acetyl-CoA, might have a large ripple effect, altering transcriptional pathways, metabolic circuits, and apoptotic sensitivity.

Regulation of N-terminal acetylation likely also occurs at the level of the acetyltransferases. Indeed, NAT complexes show differing preferences for specific N-terminal amino acid sequences (Arnesen, 2011). Furthermore, Yi et al. show that RNAi-mediated ablation of ARD1, a component of the NatA complex, sensitizes cells to apoptosis and results in the failure of a subset of apoptotic regulators to be N-terminally acetylated. Therefore, different NATs may target specific groups of proteins. Thus, taking advantage of the subtiligase approach developed by Yi et al. to identify the substrates of individual NATs should be illuminating.

Bcl-xL's antiapoptotic effect is generally attributed to suppression of mitochondrial cytochrome *c* release through inhibition of Bax/Bak. However, more than a decade ago, the Hardwick laboratory showed that Bcl-xL mutants unable to bind Bax/Bak still retain up to 80% of their antiapoptotic activity (Cheng et al., 1996). Intriguingly, Yi et al. show that these same mutants suppress acetyl-CoA levels, suggesting that altering protein N-terminal acetylation through modulation of this metabolite might contribute significantly to Bcl-xL's antiapoptotic activity.

Obviously, these data raise the question of how Bcl-xL impacts acetyl-CoA levels. Under glucose-replete conditions, citrate, a product of the tricarboxylic acid (TCA) cycle, exits the mitochondria and is converted to acetyl-CoA by ATP citrate lyase. Interestingly, Yi et al. show that stimulating acetyl-CoA production, by adding citrate to cell culture media, sensitizes cells to doxorubicin-induced death, whereas RNAi ablation of ATP citrate lyase abrogates this effect. In probing this further, Yi et al. find that citrate levels are reduced in Bcl-xL-expressing cells. Therefore, Bcl-xL may exert its effect on acetyl-CoA levels by inhibiting the production and/or export of citrate. The authors suggest that Bcl-xL might block citrate export from the mitochondria by interacting with the voltage-dependent anion channel (VDAC) (Shimizu et al., 1999; Vander Heiden et al., 2001), a component of the mitochondrial pore complex that can regulate metabolite efflux. Bcl-xL expression can also inhibit cell proliferation, which might indirectly affect acetyl-CoA levels by reducing TCA cycle activity. In this regard, it is interesting to note that the cell-cycle-delaying and antiapoptotic activities of Bcl-xL cosegregate in Bcl-xL mutants (Janumyan et al., 2003). Additionally, expression of Bcl-xL alters mitochondrial fusion/fission dynamics (Sheridan et al., 2008),

which can impact mitochondrial metabolism (reviewed in Zorzano et al., 2010).

In a variety of tumor types, Bcl-xL expression is critical for cell survival and, as such, is considered a promising therapeutic target. The work of Yi et al. raises important issues regarding the targeting of Bcl-xL and its signaling partners. For example, in tumor cells where Bcl-xL is highly expressed, how does lowering acetyl-CoA levels affect the cell's biosynthetic needs, most notably for fatty-acid synthesis? What effect does Bcl-xL have on lysine acetylation, which also requires acetyl-CoA and would likely impact metabolic pathways in cancer? Although these and other questions remain, Yi et al. provide strong evidence for a simple and elegant mechanism by which the levels of a single metabolite, acetyl-CoA, can directly regulate a cell's sensitivity to apoptosis.

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